ASSAY METHOD AND KIT THEREFOR

Field of the invention

The present invention relates to a method of quantitatively or semi-quantitatively determining an analyte in a sample, especially a high concentration analyte.

Background of the invention

For qualitative and quantitative determination of an analyte in a sample, a so-called sandwich assay is often used, wherein two receptors directed against different epitopes of the analyte are incubated with a sample containing the analyte, one of the receptors being detectable, e.g. through a label conjugated thereto. In a heterogeneous assay format, the second receptor is immobilized (e.g. coupled) to a solid phase or provided with a binder component, such as biotin, capable of binding to the solid phase, such as an avidin- or streptavidin-coated solid phase.

Especially in case the analyte is present in the sample in a high concentration, it is customary to dilute the sample before performing the assay to avoid the use of large and often costly amounts of immobilized receptor and labelled receptor, respectively, or to avoid technical difficulties where large amounts of receptors cannot be used. Such dilution is not only laborious but also introduces an additional source of error into the assay.

There is therefore a need of an assay procedure that avoids the necessity of dilution.

Summary of the invention

It is an object of the present invention to provide a method of performing a heterogeneous sandwich assay which permits the determination of even a high concentration analyte in a sample without the need to dilute the sample.

It is another object of the invention to provide a method of performing a heterogeneous sandwich assay which reduces the amounts of capturing and detection reagents used.

It is still another object of the invention to provide test kits for carrying out the method.

In one aspect of the present invention there is therefore provided a method of determining an analyte in a sample, especially a high concentration analyte found in concentrations >1 nmole/litre, comprising the steps of:

a) contacting the sample with a specified amount of a receptor which binds specifically to the analyte to form an analyte/receptor complex, said specified amount of receptor being in excess of that required to bind all analyte in the sample,

b) isolating on a solid phase, preferably a matrix such as a membrane strip, a specified fraction of the amount of receptor contacted with the analyte, including analyte/receptor complex and unreacted receptor,

c) detecting the amount of analyte/receptor complex in said isolated specified fraction, and

d) from the detected amount analyte/receptor complex, determining the concentration of analyte in the sample.

In another aspect of the present invention there is provided a test kit for determining an analyte in a sample, comprising (i) a specified amount of a receptor substance having a first part which binds specifically to the analyte, and (ii) a solid phase member having immobilized thereon a ligand which binds specifically to a second part of the receptor, the amount of said ligand on the solid phase member being less than said specified amount of the receptor substance.

In still another aspect of the present invention there is provided a test kit for determining an analyte in a sample, comprising (i) a specified amount of a receptor substance having a first part which binds specifically to the analyte, only a specified fraction of the amount of receptor substance having a second part capable of binding to a specific ligand, and (ii) a solid phase member having said specific ligand immobilized thereon.

In yet another aspect of the present invention there is provided a test kit for determining an analyte in a sample, comprising (i) a first specified amount of a receptor substance, and (ii) a solid phase member having immobilized thereon a second specified amount of the receptor substance.

While it is preferred to use the method and test kit for quantitative determination of analytes of interest, they may also be used for semi-quantitative and qualitative determinations.

Detailed description of the invention

The essence of the present invention resides in binding all analyte present in a sample to an analyte-specific receptor, isolating a minor fraction of the analyte-receptor complex formed on a solid phase, detecting the amount of isolated analyte-receptor complex, and from this detected amount of analyte on the solid phase determining the total amount of analyte in the sample. According to the invention, this may be accomplished in various ways.

In one embodiment of method of the invention, all analyte is bound by contacting the analyte-containing sample with a solution containing an excess of a first receptor (R1) which in addition to affinity to the analyte has affinity to a ligand (L), whereupon a minor fraction of the analyte-receptor complex is bound to a solid phase having the ligand (L) immobilized thereto. This binding of the minor fraction may be achieved by either (i) using a limited (specified) amount of ligand (L) to extract a fraction of the analyte-receptor complex (and unreacted receptor), or (ii) by using a first receptor (R1) only a minor (specified) fraction of which is capable of binding to the ligand (L) to extract the desired fraction of the analyte-receptor complex (and unreacted receptor). In the latter case (ii), the amount of immobilized ligand (L) is usually in excess of the amount of the first receptor capable of binding to the ligand (L). The amount of analyte/receptor complex bound to the solid phase is then detected, usually by contacting the solid phase with a detecting agent in the form of a labelled binder for the analyte, such as a labelled second receptor (R2).

In the first case (i) above, the amount of immobilized ligand (L) that can bind to the analyte-specific receptor (R1) is a specified fraction of the amount of analyte-specific receptor (R1) contacted with the sample, and therefore the ratio of detected analyte on the solid phase to the total amount of analyte in the sample will correspond to the ratio of immobilized analyte-binding ligand (L) to the total amount of added receptor (R1), thereby permitting the analyte concentration in the sample to be calculated.

In the second case (ii) above, the amount of analyte-specific receptor (R1) that can bind to immobilized ligand (L) is a specified fraction of the total amount of receptor (R1), and therefore the ratio of detected analyte on the solid phase to the total amount of analyte in the sample will correspond to the ratio of analyte-specific receptor (R1)